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April 30, 2010

Dr. Rick Sherrard Tennessee Valley Authority 1101 Market Street, PSC 1X-C Chattanooga, TN 37402

**RE: ETS PROJECT NUMBER: 6130** 

Dear Dr. Sherrard:

Enclosed are toxicity test results for samples in support of the **Kingston Fly Ash Recovery Project** received by Environmental Testing Solutions, Inc. on April 21, 2010.

All unusual observations or deviations from standard test protocols are documented on the laboratory bench sheets. If you have any questions concerning these results, please feel free to contact me.

Sincerely,

Jim Sumner

**Laboratory Director** 

CC: Dr. William Rogers, Tennessee Valley Authority



# ENVIRONMENTAL TESTING SOLUTIONS, INC. TOXICITY TEST REPORT

#### **INTRODUCTION / EXECUTIVE SUMMARY**

Report Date: April 30, 2010

ETS Project #: 6130

1. Client:

**Tennessee Valley Authority** 

2. Study:

Kingston Fossil Plant, Fly Ash Recovery Project

3. Samples Tested:

Stilling Pond Outfall 001, Emory River Dredge Plume,

**Unaffected Emory River Water (ERM 12.2)** 

4. Date Samples Received:

April 21, 2010

#### **METHODS SUMMARY** (see Appendix A for Additional Test Information)

#### Samples:

1. Sample Type:

Grab samples of Emory River Dredge Plume and ERM12.2 and 24-hour composite samples of Stilling Pond Outfall 001 were collected.

2. Sample Transportation, Storage, and Manipulation:

Samples were placed in ice chests on ice immediately after collection, where they remained during transport to Environmental Testing Solutions, Inc. by private courier. All samples were received at < 6.0°C and were refrigerated at < 6.0°C when not in use.

Aliquots of these samples, needed to prepare all test dilutions, were warmed to test temperature  $(25.0 \pm 1.0^{\circ}\text{C})$  in a warm water bath immediately prior to use.

Sufficient volumes of each dilution were prepared to split the test concentrations between the Daphnid and fathead minnow tests.

Aliquots of each dilution were also UV treated. Fish pathogens present in upstream river water have been the suspected cause of test interferences (anomalous dose response and high variability among replicates) in previous toxicity testing at the Kingston Fossil Plant. These aliquots were UV-treated through a 40-watt Smart<sup>®</sup> UV Sterilizer (manufactured by Emperor Aquatics, Inc.) for 2 to 5 minutes (dependent on sample turbidity).



### **Test Organisms:**

22.

Statistics:

# <u>Pimephales promelas</u> <u>Ceriodaphnia dubia</u>

1.Source:Aquatox, Inc.In-house Cultures2.Age:<24-hours old</td><24-hours old</td>

### **Test Conditions Summary:**

| 100000 | Araitrono Summary.        |  |  |
|--------|---------------------------|--|--|
| 1.     | Test Type/Conditions:     | Static Acute, Renewal at 48-hours              | Static Acute, Renewal at 48-hours        |
| 2.     | Test Duration:            | 96-hours                                       | 96-hours                                 |
| 3.     | Test Temperature:         | $25.0 \pm 1.0^{\circ}$ C                       | $\frac{1}{25.0 \pm 1.0^{\circ}\text{C}}$ |
| 4.     | Light Quality:            | Wide-spectrum fluorescent lighting             | Wide-spectrum fluorescent lighting       |
| 5.     | Light Intensity:          | 50 − 100 ft-c                                  | 50 - 100  ft-c                           |
| 6.     | Photoperiod:              | 16-hours light, 8-hours dark                   | 16-hours light, 8-hours dark             |
| 7.     | Test Chamber Size/Type:   | 500 mL plastic disposable cup                  | 40 mL polypropylene cups                 |
| 8.     | Test Solution Volume:     | 200 mL   | 35 mL                                    |
| 9.     | Number of Replicates:     | <u>5</u>                                       | 5  |
| 10.    | Number of Organisms       |  | _  |
|        | per Replicate:            | <u>10</u>                                      | <u>5</u>                                 |
| 11.    | Number of Organisms       |  | _  |
|        | per Test Concentration:   | <u>50</u>                                      | <u>25</u>                                |
| 12.    | Feeding regime:           | Fed newly hatched Artemia                      | Fed YWT and Selenastrum                  |
|        |                           | in holding prior to test initiation            | in holding prior to test initiation      |
|        |                           | and 2-hours prior to test solution             | and 2-hours prior to test solution       |
|        |                           | renewal at 48-hours.                           | renewal at 48-hours.                     |
| 13.    | Aeration:                 | None   | None                                     |
| 14.    | Control / Dilution Water: | <u>Unaffected Emory River Water</u>            | <b>Unaffected Emory River Water</b>      |
|        |                           | (ERM 12.2)                                     | (ERM 12.2)                               |
| 15.    | Laboratory QC:            | Moderately Hard Synthetic Water                | Moderately Hard Synthetic Water          |
| 16.    | Test Chamber Cleaning:    | None   | None None                                |
| 17.    | Test Concentrations (%):  | 100, 50, 25, 12.5, 6.25, 0 (river control),    | <u>100, 50, 25, 12.5, 6.25,</u>          |
|        |                           | MHSW   | 0 (river control), MHSW                  |
| 18.    | Sample Holding Time:      | First use $\leq$ 36-hours                      | First use $\leq$ 36-hours                |
| 19.    | Endpoints:                | Survival                                       | Survival                                 |
| 20.    | Test Acceptability        |  |  |
|        | Criteria:                 | ≥ 90% survival in river control                | ≥ 90% survival in river control          |
|        |                           | and negative control                           | and negative control                     |
| 21.    | Physical / Chemical       |  |  |
|        | Measurements:             | Alkalinity, hardness, and total residual chlor |  |
|        |                           | strength sample tested. Daily temperatures     | were measured in one replicate for       |

Alkalinity, hardness, and total residual chlorine were measured in each full-strength sample tested. Daily temperatures were measured in one replicate for each test concentration. Pre-exposure test solutions were analyzed at test initiation and at the 48-hour renewal for pH, conductivity, and dissolved oxygen. Post-exposure test solutions were analyzed daily for pH and dissolved oxygen.

Statistics were performed according to methods prescribed by EPA using ToxCalc version 5.0.23F statistical software (Tidepool Scientific

Software, McKinneyville, CA).



### **TOXICITY TEST RESULTS**

1. Sample:

Emory River Dredge Plume (see Appendix B for ToxCalc<sup>TM</sup> Statistics Reports)

Collection Date: April 20, 2010

Test Dates:

April 21 - 25, 2010

96-hour Survival:

Non-treated Sample:

Ceriodaphnia dubia: NOEC = 100% Pimephales promelas: NOEC = 100%

UV-treated Sample:

Pimephales promelas: NOEC = 100%

2. Sample:

Stilling Pond Outfall 001 (see Appendix C for ToxCalc<sup>TM</sup> Statistics Reports)

Collection Date: April 20, 2010

Test Dates:

April 21 - 25, 2010

96-hour Survival:

Non-treated Sample:

Ceriodaphnia dubia: NOEC = 100% Pimephales promelas: NOEC = 100%

UV-treated Sample:

Pimephales promelas: NOEC = 100%



# APPENDIX A ADDITIONAL TOXICITY TEST INFORMATION

#### **DEVIATIONS / MODIFICATIONS TO TEST PROTOCOL**

1. Pimephales promelas

Minnows were < 24-hours old at test initiation.

2. Ceriodaphnia dubia

None

#### **DEVIATIONS / MODIFICATIONS TO PRETEST CULTURE OR HOLDING OF TEST ORGANISMS**

1. Pimephales promelas

None

2. Ceriodaphnia dubia

None

#### PHYSICAL AND CHEMICAL METHODS

- 1. Reagents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates.
- 2. Instruments: All identification, service, and calibration information pertaining to laboratory instruments is recorded in calibration and maintenance logbooks.
- 3. Temperature was measured by SM 2550 B.
- 4. Dissolved oxygen was measured by SM 4500 O G.
- 5. The pH was measured by SM 4500 H+ B.
- 6. Conductance was measured by SM 2510 B.
- 7. Alkalinity was measured by SM 2320 B.
- 8. Total hardness was measured by SM 2340 C.
- 9. Total residual chlorine was measured by ORION Electrode Method 97-70.

#### **QUALITY ASSURANCE**

Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage, glassware preparation, test organism culturing/acquisition and acclimation, test organism handling during test, and maintaining appropriate test conditions were conducted according to the protocol as described in this report and EPA-821-R-02-012. Any known deviations were noted during the study and are reported herein.

#### **REFERENCE TOXICANT TESTS** (reference toxicant data is available upon request)

1. Test Type: 96-hour acute tests with results expressed as LC<sub>50</sub> values in g/L KCl or NaCl.

2. Standard Toxicant: Potassium Chloride (KCl crystalline) for *Pimephales promelas*.

Sodium Chloride (NaCl crystalline) for Ceriodaphnia dubia.

3. Dilution Water Used: Moderately hard synthetic water.

4. Statistics: ToxCalc software Version 5.0 was used for statistical analyses.

#### **REFERENCES**

- 1. USEPA. Short-Term Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, EPA-821-R-02-012 (October 2002).
- 2. Standard Methods for the Examination of Water and Wastewater, 21st Edition, 2005.
- 3. Quality Assurance Program: Standard Operating Procedures, Environmental Testing Solutions, Inc.



## APPENDIX B

ToxCalc<sup>TM</sup> v5.0.23F Statistics Report for *Ceriodaphnia dubia* and *Pimephales promelas* 96-hour Acute Toxicity Tests of TVA Kingston Fossil Plant, <u>Emory River Dredge Plume</u> April 21 – 25, 2010

Environmental Testing Solutions, Inc. Project # 6130



# Ceriodaphnia dubia 96-Hour Acute Toxicity Test for Non-treated Emory River Dredge Plume April $21-25,\,2010$

|               |               |             |             |                        | Acute Daphnid Tes | st-96 Hr Survival |                               |
|---------------|---------------|-------------|-------------|------------------------|-------------------|-------------------|-------------------------------|
| Start Date:   | 4/21/2010     | T           | est ID:     | 6130                   |                   | Samp le ID:       | KIF, Emory River Dredge Plume |
| End Date:     | 4/25/2010     | L           | ab ID:      | ETS-Envir. Tes         | sting Sol.        | Sample Type:      | Non-treated Grab              |
| Sample Date:  | 4/20/2010     | P           | rotocol:    | ACUTE-EPA-821-R-02-012 |                   | Test Species:     | CD-Ceriodaphnia dubia         |
| Comments:     | 1 Dredge Plui | me grab sam | ple for day | 0 (initiation)         | and day 2 (renewa | al)               | -                             |
| Conc-%        | 1             | 2           | 3           | 4                      | 5                 |                   |                               |
| M HSW-Control | 1.0000        | 1.0000      | 1.0000      | 1.0000                 | 1.0000            |                   |                               |
| ERM-Control   | 1.0000        | 1.0000      | 1.0000      | 1.0000                 | 1.0000            |                   |                               |
| 6.25          | 1.0000        | 1.0000      | 1.0000      | 1.0000                 | 1.0000            |                   |                               |
| 12.5          | 1.0000        | 1.0000      | 1.0000      | 1.0000                 | 1.0000            |                   |                               |
| 25            | 1.0000        | 1.0000      | 1.0000      | 1.0000                 | 1.0000            |                   |                               |
| 50            | 1.0000        | 1.0000      | 1.0000      | 1.0000                 | 1.0000            |                   |                               |
| 100           | 1.0000        | 1.0000      | 1.0000      | 1.0000                 | 1.0000            |                   |                               |

|              |        | _       |        | Transform | : Arcsin Squa | Transform: Arcsin Square Root |   |       |          |  |  |
|--------------|--------|---------|--------|-----------|---------------|-------------------------------|---|-------|----------|--|--|
| Conc-%       | M ean  | N-M ean | M ean  | M in      | M ax          | CV%                           | N | Sum   | Critical |  |  |
| MHSW-Control | 1.0000 | 1.0000  | 1.3453 | 1.3453    | 1.3453        | 0.000                         | 5 | •     |          |  |  |
| ERM-Control  | 1.0000 | 1.0000  | 1,3453 | 1.3453    | 1.3453        | 0.000                         | 5 |       |          |  |  |
| 6.25         | 1.0000 | 1.0000  | 1.3453 | 1.3453    | 1.3453        | 0.000                         | 5 | 27.50 | 16.00    |  |  |
| 12.5         | 1.0000 | 1.0000  | 1.3453 | 1.3453    | 1.3453        | 0.000                         | 5 | 27.50 | 16.00    |  |  |
| 25           | 1.0000 | 1.0000  | 1.3453 | 1.3453    | 1.3453        | 0.000                         | 5 | 27.50 | 16.00    |  |  |
| 50           | 1.0000 | 1.0000  | 1.3453 | 1.3453    | 1.3453        | 0.000                         | 5 | 27.50 | 16.00    |  |  |
| 100          | 1.0000 | 1,0000  | 1.3453 | 1.3453    | 1,3453        | 0.000                         | 5 | 27.50 | 16.00    |  |  |

| Auxiliary Tests                           |                     | -    |     |    | Statistic | Critical  | Skew | Kurt |
|---|---------------------|------|-----|----|-----------|-----------|------|------|
| Shapiro-Wilk's Test indicates normal dist | ribution (p > 0.01  | )    |     |    | 1         | 0.9       |      |      |
| Equality of variance cannot be confirmed  |                     |      |     |    |           |           |      |      |
| The control means are not significantly d | ifferent (p = 1.00) |      |     |    | 0         | 2.3060041 |      |      |
| Hypothesis Test (1-tail, 0.05)            | NOEC                | LOEC | ChV | TU |           |           |      |      |
| Steel's Many-One Rank Test                | 100                 | >100 |     | 1  |           |           |      |      |
| Treatments vs ERM-Control                 |                     |      |     |    |           |           |      |      |

# Pimephales promelas 96-Hour Acute Toxicity Test for Non-treated Emory River Dredge Plume April 21-25,2010

|               |               |             |             | Acut           | e Fathead Minnow  | Test-96 Hr Survival |                               |
|---------------|---------------|-------------|-------------|----------------|-------------------|---------------------|-------------------------------|
| Start Date:   | 4/21/2010     | T           | est ID:     | 6130           |                   | Sample ID:          | KIF, Emory River Dredge Plume |
| End Date:     | 4/25/2010     | L           | ab ID:      | ETS-Envir. Tes | ting Sol.         | Sample Type:        | Non-treated Grab              |
| Sample Date:  | 4/20/2010     | Pı          | otocol:     | ACUTE-EPA-     | 821-R-02-012      | Test Species:       | PP-Pimephales promelas        |
| Comments:     | 1 Dredge Plui | ne grab sam | ple for day | 0 (initiation) | and day 2 (renewa | al)                 | -                             |
| Conc-%        | 1             | 2           | 3           | 4              | 5                 |                     |                               |
| M HSW-Control | 1.0000        | 1.0000      | 1.0000      | 1.0000         | 1.0000            |                     |                               |
| ERM-Control   | 1.0000        | 1.0000      | 1.0000      | 1.0000         | 1.0000            |                     |                               |
| 6.25          | 1.0000        | 1.0000      | 1.0000      | 1.0000         | 1.0000            |                     |                               |
| 12.5          | 1,0000        | 1.0000      | 1.0000      | 1.0000         | 1.0000            |                     |                               |
| 25            | 1.0000        | 1.0000      | 1.0000      | 1,0000         | 1.0000            |                     |                               |
| 50            | 1.0000        | 1.0000      | 1.0000      | 1,0000         | 1.0000            |                     |                               |
| 100           | 1.0000        | 1.0000      | 1.0000      | 1.0000         | 1.0000            |                     |                               |

|              |        | _       |        | Transform | : Arcsin Squa | re Root |   | Rank  | 1-Tailed |
|--------------|--------|---------|--------|-----------|---------------|---------|---|-------|----------|
| Conc-%       | M ean  | N-M ean | M ean  | M in      | Max           | CV%     | N | Sum   | Critical |
| MHSW-Control | 1.0000 | 1.0000  | 1.4120 | 1.4120    | 1.4120        | 0.000   | 5 |       |          |
| ERM-Control  | 1.0000 | 1.0000  | 1.4120 | 1.4120    | 1.4120        | 0.000   | 5 |       |          |
| 6,25         | 1.0000 | 1.0000  | 1.4120 | 1.4120    | 1.4120        | 0.000   | 5 | 27.50 | 16.00    |
| 12.5         | 1.0000 | 1.0000  | 1.4120 | 1.4120    | 1.4120        | 0.000   | 5 | 27.50 | 16.00    |
| 25           | 1.0000 | 1.0000  | 1.4120 | 1.4120    | 1.4120        | 0.000   | 5 | 27.50 | 16.00    |
| 50           | 1.0000 | 1.0000  | 1.4120 | 1.4120    | 1.4120        | 0.000   | 5 | 27.50 | 16.00    |
| 100          | 1.0000 | 1.0000  | 1.4120 | 1.4120    | 1.4120        | 0.000   | 5 | 27.50 | 16.00    |

| Auxiliary Tests                            | ,                     |      |     |    | Statistic | Critical  | Skew | Kurt |
|--|-----------------------|------|-----|----|-----------|-----------|------|------|
| Shapiro-Wilk's Test indicates normal dist  | ribution (p > 0.01    | )    |     |    | 1         | 0.9       |      |      |
| Equality of variance cannot be confirmed   |                       |      |     |    |           |           |      |      |
| The control means are not significantly di | ifferent $(p = 1.00)$ |      |     |    | 0         | 2.3060041 |      |      |
| Hypothesis Test (1-tail, 0.05)             | NOEC                  | LOEC | ChV | TU |           |           |      |      |
| Steel's Many-One Rank Test                 | 100                   | >100 |     | 1  |           |           |      |      |
| Treatments vs ERM-Control                  |                       |      |     |    |           |           |      |      |



# Pimephales promelas 96-Hour Acute Toxicity Test for UV-treated Emory River Dredge Plume April 21-25,2010

| Rank Test       |  | 100          | >100  |  | 1  |               |   |  |             |  |
|-----------------|--|--------------|---|--|--|---------------|---|--|-------------|--|
|                 |  | 4            | LOEC  | ChV  | TU   | -             |   |  |             |  |
|                 |  | (p = 1.00)   |   |  |  | 0             |   | 2.3060041  |             |  |
|                 |  | n (b > 0.01) | ,   |  |  | 1             |   | 0.5  |             |  |
| t indicates non | mal distributio  | n (n > 0.01) |   |  |  |               |   |  | SKCW        | Kui  |
|                 |  |              |   |  |  | Statistic     |   | Critical   | Skew        | Kurt   |
|                 |  |              |   |  |  |               |   |  |             |  |
| 1.0000          | 1.0000   | 1.4120       | 1.4120  | 1.4120   | 0.000  | 5             | 27.50   | 16.00  |             |  |
| 1.0000          | 1.0000   | 1.4120       | 1.4120  | 1.4120   | 0.000  | 5             | 27.50   | 16.00  |             |  |
| 1.0000          | 1.0000   | 1.4120       | 1.4120  | 1.4120   | 0.000  | 5             | 27.50   | 16.00  |             |  |
| 1.0000          | 1.0000   | 1.4120       | 1.4120  | 1.4120   | 0.000  |               | 27.50   | 16.00  |             |  |
| 1.0000          | 1.0000   | 1.4120       | 1.4120  | 1.4120   | 0.000  |               | 27.50   | 16.00  |             |  |
| 1.0000          | 1.0000   | 1.4120       | 1.4120  | 1.4120   |  |               |   |  |             |  |
| 1.0000          | 1.0000   | 1,4120       | 1.4120  | 1.4120   |  |               |   |  |             |  |
| M ean           | N-M ean  | Mean         | Min   |  |  | N             |   |  |             |  |
|                 |  |              | Transform   | Arcsin Squar   | e Root   |               | Rank  | 1-Tailed   |             |  |
| 1,000           | 2,0000   | 1.0000       | 1.0000  | 1.0000   |  |               |   |  |             |  |
|                 |  |              |   |  |  |               |   |  |             |  |
|                 |  |              |   |  |  |               |   |  |             |  |
|                 |  |              |   |  |  |               |   |  |             |  |
|                 |  |              |   |  |  |               |   |  |             |  |
|                 |  |              |   |  |  |               |   |  |             |  |
|                 |  |              |   |  |  |               |   |  |             |  |
| 1 1 2000        |  |              | · · · · · · · · · · · · · · · · · · ·   |  |  |               |   |  |             |  |
| 1 Dredge Pl     |  |              |   |  | enewal)  |               |   |  |             |  |
|                 |  |              |   |  | _  | Test Species: | :   | PP-Pimephales prom   | elas        |  |
|                 |  |              |   | -  |  | Sample Type:  |   |  |             |  |
| 4/21/2010       | 7  | Γest ID:     | 6130  |  |  | Sample ID:    |   | KIF, Emory River D   | redge Plume |  |
|                 | 4/25/2010 4/20/2010 1 Dredge Pl 1 1.0000 | 4/25/2010    | 4/25/2010         Lab ID:           4/20/2010         Protocol:           1 Dredge Plume grab sample for day         1         2         3           1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000           Mean         N-Mean         Mean           1.0000         1.0000         1.4120           1.0000         1.0000         1.4120           1.0000         1.0000         1.4120           1.0000         1.0000         1.4120           1.0000         1.0000         1.4120           1.0000         1.0000         1.4120           1.0000         1.0000         1.4120           1.0000         1.0000         1.4120           1.0000         1.0000         1.4120           1.0000         1.0000         1.4120           1.0000         1.0000         1.4120           1.0000         1.0000         1.4120 | 4/20/2010         Protocol:         ACUTE-EPA           1 Dredge Plume grab sample for day 0 (initiation)           1         2         3         4           1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.4120         1.4120           1.0000         1.0000         1.4120         1.4120           1.0000         1.0000         1.4120         1.4120           1.0000         1.0000         1.4120         1.4120           1.0000         1.0000         1.4120         1.4120           1.0000         1.0000         1.4120         1.4120           1.0000         1.0000         1.4120         1.4120           1.0000         1.0000         1.4120         1.4120           1.0000 | 4/25/2010         Lab ID:         ETS-Envir. Testing Sol.           4/20/2010         Protocol:         ACUTE-EPA-821-R-02-01:           1 Dredge Plume grab sample for day 0 (initiation) and day 2 (residual)         1         2         3         4         5           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.4120         1.4120           1.0000         1.0000         1.4120         1.4120         1.4120         1.4120           1.0000         1.0000         1.4120         1.4120         1.4120         1.4120           1.0000         1.0000         1.4120         1.4120         1.4120 | A/25/2010     | 4/25/2010         Lab ID:         ETS-Envir. Testing Sol.         Samp le Type:           4/20/2010         Protocol:         ACUTE-EPA-821-R-02-012         Test Species:           1 Dredge Plume grab sample for day 0 (initiation) and day 2 (renewal)           1         2         3         4         5           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         5           1.0000         1.0000         1.4120         1.4120         0.000         5           1.0000         1.0000         1.4120         1.4120         1.4120         0.000         5           1.0000         1.0000         1.4120 <td>4/25/2010         Lab ID: ETS-Envir. Testing Sol. Samp le Type:           4/20/2010         Protocol: ACUTE-EPA-821-R-02-012 Test Species:           1 Dredge Plume grab sample for day 0 (initiation) and day 2 (renewal)           1         2         3         4         5           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.4120         1.4120         0.000         5           1.0000         1.0000         1.4120         1.4120         0.000         5         27.50           1.0000         1.0000         1.4120         1.4120         0.000         5         27.50           1.0000         1.0000         <t< td=""><td>  A/25/2010</td><td>4/25/2010         Lab ID:         ETS-Envir. Testing Sol.         Sample Type:         UV-treated Grab           4/20/2010         Protocol:         ACUTE-EPA-821-R-02-012         Test Species:         PP-Pimephales promelas           1 Dredge Plume grab sample for day 0 (initiation) and day 2 (renewal)           1         2         3         4         5           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.4120         1.4120         0.000         5           1.0000         1.0000         1.4120         1.4120         0.000         5</td></t<></td> | 4/25/2010         Lab ID: ETS-Envir. Testing Sol. Samp le Type:           4/20/2010         Protocol: ACUTE-EPA-821-R-02-012 Test Species:           1 Dredge Plume grab sample for day 0 (initiation) and day 2 (renewal)           1         2         3         4         5           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.4120         1.4120         0.000         5           1.0000         1.0000         1.4120         1.4120         0.000         5         27.50           1.0000         1.0000         1.4120         1.4120         0.000         5         27.50           1.0000         1.0000 <t< td=""><td>  A/25/2010</td><td>4/25/2010         Lab ID:         ETS-Envir. Testing Sol.         Sample Type:         UV-treated Grab           4/20/2010         Protocol:         ACUTE-EPA-821-R-02-012         Test Species:         PP-Pimephales promelas           1 Dredge Plume grab sample for day 0 (initiation) and day 2 (renewal)           1         2         3         4         5           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.4120         1.4120         0.000         5           1.0000         1.0000         1.4120         1.4120         0.000         5</td></t<> | A/25/2010   | 4/25/2010         Lab ID:         ETS-Envir. Testing Sol.         Sample Type:         UV-treated Grab           4/20/2010         Protocol:         ACUTE-EPA-821-R-02-012         Test Species:         PP-Pimephales promelas           1 Dredge Plume grab sample for day 0 (initiation) and day 2 (renewal)           1         2         3         4         5           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.0000         1.0000         1.0000         1.0000           1.0000         1.0000         1.4120         1.4120         0.000         5           1.0000         1.0000         1.4120         1.4120         0.000         5 |



### APPENDIX B

ToxCalc<sup>™</sup> v5.0.23F Statistics Report for *Ceriodaphnia dubia* and *Pimephales promelas* 96-hour Acute Toxicity Tests of TVA Kingston Fossil Plant, <u>Stilling Pond Outfall 001</u> April 21 – 25, 2010

Environmental Testing Solutions, Inc. Project # 6130



## Ceriodaphnia dubia 96-Hour Acute Toxicity Test for Non-treated Stilling Pond Outfall 001 April 21 – 25, 2010

|                      |                 |                |               |               | Acute Daphn    | id Test-96 I | Hr Survival   |       |                      |            |       |
|----------------------|-----------------|----------------|---------------|---------------|----------------|--------------|---------------|-------|----------------------|------------|-------|
| Start Date:          | 4/21/2010       | -              | Γest ID:      | 6130          |                | -            | Sample ID:    |       | KIF, Stilling Pond O | utfall 001 |       |
| End Date:            | 4/25/2010       | 1              | Lab ID:       | ETS-Envir. To | esting Sol.    |              | Sample Type:  |       | Non-treated 24-hour  | Composite  |       |
| Sample Date:         | 4/20/2010       | I              | Protocol:     | ACUTE-EPA     | -821-R-02-012  | 2            | Test Species: |       | CD-Ceriodaphnia du   | ıbia       |       |
| Comments:            | 1 Stilling Po   | ond Effluent   | composite s   | ample for day | 0 (initiation  | and day 2    | (renewal)     |       | •                    |            |       |
| Conc-%               | 1               | 2              | 3             | 4             | 5              |              |               |       |                      |            |       |
| MHSW-Control         | 1.0000          | 1.0000         | 1.0000        | 1.0000        | 1.0000         |              |               |       |                      |            |       |
| ERM -Control         | 1.0000          | 1.0000         | 1.0000        | 1.0000        | 1.0000         |              |               |       |                      |            |       |
| 6.25                 | 1.0000          | 1.0000         | 1.0000        | 1.0000        | 1.0000         |              |               |       |                      |            |       |
| 12.5                 | 1.0000          | 1.0000         | 1.0000        | 1.0000        | 1.0000         |              |               |       |                      |            |       |
| 25                   | 1.0000          | 1.0000         | 1.0000        | 1.0000        | 1.0000         |              |               |       |                      |            |       |
| 50                   | 1.0000          | 1.0000         | 1.0000        | 1.0000        | 1.0000         |              |               |       |                      |            |       |
| 100                  | 1.0000          | 1.0000         | 1.0000        | 1.0000        | 1.0000         |              |               |       |                      |            |       |
|                      |                 |                |               |               |                |              |               |       |                      |            |       |
|                      |                 |                | _             |               |                |              |               |       |                      |            |       |
|                      |                 |                |               |               | : Arcsin Squar |              |               | Rank  | 1-Tailed             |            |       |
| Conc-%               | Mean            | N-Mean         | M ean         | M in          | Max            | CV%          | N             | Sum   | Critical             |            |       |
| MHSW-Control         | 1.0000          | 1.0000         | 1.3453        | 1.3453        | 1.3453         | 0.000        | 5             |       |                      |            |       |
| ERM -Control         | 1.0000          | 1.0000         | 1.3453        | 1.3453        | 1.3453         | 0.000        | 5             |       |                      |            |       |
| 6.25                 | 1.0000          | 1.0000         | 1.3453        | 1.3453        | 1.3453         | 0.000        | 5             | 27.50 | 16.00                |            |       |
| 12.5                 | 1.0000          | 1.0000         | 1.3453        | 1.3453        | 1.3453         | 0.000        | 5             | 27.50 | 16.00                |            |       |
| 25                   | 1.0000          | 1.0000         | 1.3453        | 1.3453        | 1.3453         | 0.000        | 5             | 27.50 | 16.00                |            |       |
| 50                   | 1.0000          | 1.0000         | 1.3453        | 1.3453        | 1.3453         | 0.000        | 5             | 27.50 | 16.00                |            |       |
| 100                  | 1.0000          | 1.0000         | 1.3453        | 1.3453        | 1.3453         | 0.000        | 5             | 27.50 | 16.00                |            |       |
| Auxiliary Tests      |                 |                |               |               |                |              | Statistic     |       | Critical             | Skew       | Kurt  |
| Shapiro-Wilk's Tes   | t indicates nor | mal distributi | on (n > 0.01  | 1             |                |              | 1             |       | 0.9                  | SRCW       | ixuit |
| Equality of variance |                 |                | (P - 0.01     | ,             |                |              | •             |       | 3.2                  |            |       |
| The control means    |                 |                | it (p = 1.00) |               |                |              | 0             |       | 2.3060041            |            |       |
| Hypothesis Test (    |                 |                | NOEC          | LOEC          | ChV            | TU           | -             |       |                      |            |       |
| Steel's Many-On      |                 |                | 100           | >100          |                | 1            | 77            | **    |                      |            |       |
| Treatments vs ER     |                 |                |               |               |                |              |               |       |                      |            |       |

## *Pimephales promelas* 96-Hour Acute Toxicity Test for Non-treated Stilling Pond Outfall 001 April 21 – 25, 2010

|              |                |              |            | Acut           | te Fathead Minnow  | Test-96 Hr Survival |                                |
|--------------|----------------|--------------|------------|----------------|--------------------|---------------------|--------------------------------|
| Start Date:  | 4/21/2010      | T            | est ID:    | 6130           |                    | Sample ID:          | KIF, Stilling Pond Outfall 001 |
| End Date:    | 4/25/2010      | L            | ab ID:     | ETS-Envir. Tes | ting Sol.          | Sample Type:        | Non-treated 24-hour Composite  |
| Sample Date: | 4/20/2010      | P            | rotocol:   | ACUTE-EPA-     | 821-R-02-012       | Test Species:       | PP-Pimephales promelas         |
| Comments:    | 1 Stilling Por | d Effluent c | omposite s | ample for day  | 0 (initiation) and | day 2 (renewal)     | · ·                            |
| Conc-%       | 1              | 2            | 3          | 4              | 5                  |                     |                                |
| MHSW-Control | 1,0000         | 1.0000       | 1.0000     | 1,0000         | 1,0000             |                     |                                |
| ERM -Control | 1.0000         | 1.0000       | 1.0000     | 1.0000         | 1.0000             |                     |                                |
| 6.25         | 1.0000         | 1.0000       | 1.0000     | 1.0000         | 1.0000             |                     |                                |
| 12.5         | 1.0000         | 1.0000       | 1.0000     | 1.0000         | 1.0000             |                     |                                |
| 25           | 1.0000         | 1.0000       | 1.0000     | 1.0000         | 1.0000             |                     |                                |
| 50           | 1.0000         | 1.0000       | 1.0000     | 1.0000         | 1.0000             |                     |                                |
| 100          | 1.0000         | 1.0000       | 1.0000     | 1.0000         | 1.0000             |                     |                                |

|              |        |        |        | Transform | : Arcsin Squa | re Root |   | Rank  | 1-Tailed |  |
|--------------|--------|--------|--------|-----------|---------------|---------|---|-------|----------|--|
| Conc-%       | Mean   | N-Mean | M ean  | M in      | Max           | CV%     | N | Sum   | Critical |  |
| MHSW-Control | 1.0000 | 1.0000 | 1.4120 | 1.4120    | 1.4120        | 0.000   | 5 |       |          |  |
| ERM-Control  | 1.0000 | 1.0000 | 1.4120 | 1.4120    | 1.4120        | 0.000   | 5 |       |          |  |
| 6.25         | 1.0000 | 1.0000 | 1.4120 | 1.4120    | 1.4120        | 0.000   | 5 | 27.50 | 16.00    |  |
| 12.5         | 1.0000 | 1.0000 | 1.4120 | 1.4120    | 1.4120        | 0.000   | 5 | 27.50 | 16.00    |  |
| 25           | 1.0000 | 1,0000 | 1.4120 | 1.4120    | 1.4120        | 0.000   | 5 | 27.50 | 16.00    |  |
| 50           | 1.0000 | 1.0000 | 1.4120 | 1.4120    | 1.4120        | 0.000   | 5 | 27.50 | 16.00    |  |
| 100          | 1.0000 | 1.0000 | 1.4120 | 1.4120    | 1.4120        | 0.000   | 5 | 27.50 | 16.00    |  |

| Auxiliary Tests                         |                        |      |     |    | Statistic | Critical  | Skew | Kurt |
|---|------------------------|------|-----|----|-----------|-----------|------|------|
| Shapiro-Wilk's Test indicates normal di | stribution $(p > 0.0)$ | 1)   |     |    | 1         | 0.9       |      |      |
| Equality of variance cannot be confirme | d                      |      |     |    |           |           |      |      |
| The control means are not significantly | different (p = 1.00)   | )    |     |    | 0         | 2.3060041 |      |      |
| Hypothesis Test (1-tail, 0.05)          | NOEC                   | LOEC | ChV | TU |           |           |      |      |
| Steel's Many-One Rank Test              | 100                    | >100 |     | 1  |           |           |      |      |
| Treatments vs ERM-Control               |                        |      |     |    |           |           |      |      |



# Pimephales promelas 96-Hour Acute Toxicity Test for UV-treated Stilling Pond Outfall 001 April 21-25, 2010

|   |                               |              |             | Acı   | ite Fathead M | innow Test      | -96 Hr Surviva | d     |                                |      |      |
|---|-------------------------------|--------------|-------------|---|---------------|-----------------|----------------|-------|--------------------------------|------|------|
| Start Date:   | 4/21/2010                     |              | Test ID:    | 6130  |               |                 | Sample ID:     |       | KIF, Stilling Pond Outfall 001 |      |      |
| End Date:   | 4/25/2010                     | Lab ID:      |             | ETS-Envir. Testing Sol.<br>ACUTE-EPA-821-R-02-012 |               |                 | Sample Type:   |       | UV-treated 24-hour Composite   |      |      |
| Sample Date:  | 4/20/2010                     |              | Protocol:   |   |               | ? Test Species: |                |       | omelas                         |      |      |
| Comments:   | 1 Stilling P                  | ond Effluent | composite s | ample for day                                     | 0 (initiation | ) and day 2     | (renewal)      |       |                                |      |      |
| Conc-%  | 1                             | 2            | 3           | 4   | 5             |                 |                |       |                                |      |      |
| MHSW-Control  | 1.0000                        | 1.0000       | 1.0000      | 1.0000  | 1.0000        |                 |                |       |                                |      |      |
| ERM-Control   | 1.0000                        | 1.0000       | 1.0000      | 1.0000  | 1.0000        |                 |                |       |                                |      |      |
| 6.25  | 1.0000                        | 1.0000       | 1.0000      | 1.0000  | 1.0000        |                 |                |       |                                |      |      |
| 12.5  | 1.0000                        | 1.0000       | 1.0000      | 1.0000  | 1.0000        |                 |                |       |                                |      |      |
| 25  | 1.0000                        | 1.0000       | 1.0000      | 1.0000  | 1.0000        |                 |                |       |                                |      |      |
| 50  | 1.0000                        | 1.0000       | 1.0000      | 1.0000  | 1.0000        |                 |                |       |                                |      |      |
| 100   | 1.0000                        | 1.0000       | 1.0000      | 1.0000  | 1,0000        |                 |                |       |                                |      |      |
|   |                               |              |             |   |               |                 |                |       |                                |      |      |
|   | Transform: Arcsin Square Root |              |             |   |               |                 |                | Rank  | 1-Tailed                       |      |      |
| Conc-%  | Mean                          | N-Mean       | M ean       | M in  | Max           | CV%             | N              | Sum   | Critical                       |      |      |
| MHSW-Control  |                               | 1.0000       | 1.4120      | 1.4120  | 1.4120        | 0.000           | 5              |       |                                |      |      |
| ERM -Control  |                               | 1.0000       | 1.4120      | 1.4120  | 1.4120        | 0.000           | 5              |       |                                |      |      |
| 6.25  | 1.0000                        | 1.0000       | 1.4120      | 1.4120  | 1.4120        | 0.000           | 5              | 27.50 | 16.00                          |      |      |
| 12.5  | 1.0000                        | 1.0000       | 1.4120      | 1.4120  | 1.4120        | 0.000           | 5              | 27.50 | 16.00                          |      |      |
| 25  | 1.0000                        | 1.0000       | 1.4120      | 1.4120  | 1.4120        | 0.000           | 5              | 27.50 | 16.00                          |      |      |
| 50  | 1.0000                        | 1.0000       | 1,4120      | 1.4120  | 1.4120        | 0.000           | 5              | 27.50 | 16.00                          |      |      |
| 100   | 1.0000                        | 1.0000       | 1.4120      | 1.4120  | 1.4120        | 0.000           | 5              | 27.50 | 16.00                          |      |      |
| uxiliary Tests  |                               |              |             |   |               |                 | Statistic      |       | Critical                       | Skew | Kurt |
| Shap iro-Wilk's Test indicates normal distribution (p > 0.01) |                               |              |             |   |               | 1               |                | 0.9   |                                |      |      |
| quality of variance   | e cannot be co                | nfirmed      | •           | •   |               |                 |                |       |                                |      |      |
| The control means are not significantly different (p = 1.00)  |                               |              |             |   |               |                 | 0              |       | 2.3060041                      |      |      |
| Hypothesis Test (1-tail, 0.05) NOEC                           |                               |              | LOEC        | ChV   | TU            |                 |                |       |                                |      |      |
| Steel's Many-One Rank Test 100                                |                               |              | >100        |   | 1             |                 |                |       |                                |      |      |
| reatments vs EI   | RM-Control                    |              |             |   |               |                 |                |       |                                |      |      |